

CERTIFICATE OF MAILING

I hereby certify that this correspondence (along with any paper referred to as being attached or enclosed) is being submitted via the USPTO EFS Filing System; Mail Stop Amendment; Commissioner for Patents; P.O. Box 1450; Alexandria, VA 22313-1450.

Date: December 30, 2009

Rebecca A. Bellas
Rebecca A. Bellas

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application

Applicant:	Kusama	:	Art Unit:	1637
		:		
Serial No.:	10/826,119	:	Examiner:	Suryaprabha Chunduru
		:		
Filed:	April 16, 2004	:		
		:		
Title:	OLIGONUCLEOTIDE SEQUENCES THAT IDENTIFY SPECIES OF ANIMAL			

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

I, Dr. Koichi Kadowaki, declare and say as follows:

I am an inventor of the claims of the above-identified patent application. I hold a Ph.D. degree in plant molecular biology awarded in 1989 from Kyoto University. I have conducted molecular biology research continuously since 1984 at the following professional positions:

1984 National Institute of Agrobiological Resources (Japan)/Researcher

1990 North Carolina State University/Post Doctoral Fellow
1991 National Institute of Agrobiological Resources (Japan)/Researcher
1997 Agency of Science and Technology, Section of Life Science (Japan)
1998 National Institute of Agrobiological Sciences (Japan)/Head of Laboratory
2006 Headquarters (Secretary of Research Council) Ministry of Agriculture, Forestry
and Fisheries (Japan)/General Officer
2009 National Institute of Agrobiological Sciences (Japan)/Research Director-General

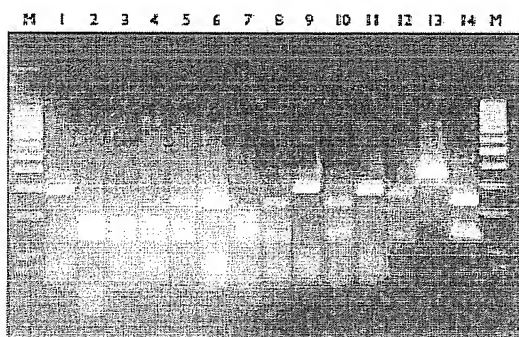
Before the oligonucleotide primers and methods disclosed in the above-referenced patent application, the only known method for determining the species origin of a target DNA in a food sample relied upon PCR followed by creation of a restriction map by RFLP (restriction fragment length polymorphism). RFLP techniques involve observing many bands formed on an electrophoretic gel created by endonuclease restriction of a PCR product. Therefore, the technician performing the determination must identify the restriction digest DNA bands among many such bands by comparison with a molecular weight ladder.

Fig. 1, below, shows exemplary results for PCR followed by RFLP using the BIOFOOD Identification Kit (Funakoshi (Japan)). The BIOFOOD Identification Kit was the most common kit for detecting animal species-derived DNA in 1999 (the year of the Lowe et al publication); however, sales of the BIOFOOD Identification Kit have now stopped.

The BIOFOOD Identification Kit is for the identification of animal species for animal derived components in a sample. First, the primer pair against a sequence common to vertebrates is used to perform PCR, and then the amplified DNA is digested with three restriction enzymes and subjected to gel electrophoresis. Animal species for animal-derived components in the sample are identified based on the electrophoretic patterns of the RFLP. That is, the PCR product formed using the primer pair against a sequence common to vertebrates in PCR is similar in all species; restriction digest is needed to identify the source of the PCR product. Sensitivity is from about 0.5 to 5% or

more of the sample. For a sample containing components derived from three or more animal species, it may be difficult to interpret the electrophoretic pattern.

Fig. 1: Exemplary Identification of Animal Species using PCR followed by RFLP.



Lane 1: buffalo; Lane 2: rabbit; Lane 3: horse; Lane 4: roe deer; Lane 5: cattle; Lane 6: Goat; Lane 7: deer; Lane 8: emu; Lane 9: red kangaroo; Lane 10: ostrich; Lane 11: cat; lane 12: dog; lane 13: muse; lane 14: human; lane M: 100 bp size marker. Source: BIOFOOD Identification Kit product information sheet.

As can be observed in Fig. 1, the result for each lane is very complex, which is compounded if a sample contains DNA from multiple species. On the contrary, the oligonucleotide primers and methods disclosed in the above-referenced patent application produce only a single band by PCR that is either present or not present depending upon the existence of a target DNA from a specific species or group of species (i.e., ruminants) in a sample. Thus, interpretation of the results is greatly simplified.

A product information sheet for the BIOFOOD Identification Kit from Funakoshi in Japanese is attached as Appendix 1 along with a partial English translation. The partial English Translation includes a translation of the legend of Fig. 1.

As shown in Figs. 6 and 7 in the Specification of the above-referenced patent application, only a single band can be detected in a single lane of an electrophoretic gel, therefore, we can clearly and easily recognize the species origin by one view of the gel without specific comparison to a DNA weight ladder. This is an unexpected utility of the

primers described in the Specification. In addition, the claimed primer pairs can detect the target DNA as the same size band regardless of animal species, which serves as additional evidence that a positive result is not due to error. At present, the detection method using the claimed primer pairs is an official method authorized by Ministry of Agriculture, Forestry and Fisheries of Japan, listed in "The Official Methods of Feed Analysis", and widely utilized for detecting trace amounts of ruminant-derived DNA samples. The users of the claimed primer pairs are private enterprises, governmental agencies, and academic researchers.

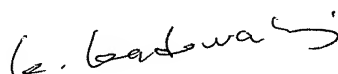
Furthermore, extensive and laborious experimentation was required to obtain the claimed primer pairs. Appendixes 2 and 3 illustrate that the claimed primer pairs were obtained from extensive experimentation. As shown in Appendix 2, we have examined various combinations of primers for PCR for specific detection of ruminant-derived DNA. In each nucleotide sequence shown as "rumicon" or cases 1 to 8 in Appendix 2, a pair of boxes shows the regions of the ATPase8 gene where a primer pair binds in PCR in each experiment correspondingly labelled "rumicon" or cases 1 to 8 in Appendix 3. The "rumicon" is the claimed primer pair, Fpr-F and Fpr-R (SEQ ID NOS. 5 and 6). The cases 1 to 8 are the primer pairs which bind to regions near the region where the claimed primer pair binds. Using the primer pairs which were designed based on the boxed regions in Appendix 2, PCR was performed on various animal-derived DNA samples according to the same procedure as Example 3 in the Specification of the above-referenced patent application. The results are shown in Appendix 3.

In cases 1 to 7, some of the non-ruminant-derived DNAs were detected and some ruminant-derived DNAs could not be detected. In case 8, although sheep-derived DNA was detected, cattle-, goat-, or deer-derived DNA was not detected. In addition, in case 8, DNA derived from swine, which is not a ruminant, showed a positive band. In case 2, goat and deer (ruminants) are detected along with non-ruminants pig and horse, all with PCR products of approximately the same length. In case 1, ruminant-derived DNA from sheep and goat were not detected, and in case 6, ruminant-derived DNA from cattle (cow) and sheep were not detected. In case 2, which successfully detected sheep- and

goat-derived DNA, ruminant-derived DNA from cattle was not detected and ruminant-derived DNA from deer produced numerous bands rather than a single band. On the contrary, as show in the "rumicon" example of Appendix 3, which is the same as Fig. 7 of the Specification of the above-referenced patent application, the ruminant-derived DNAs could be detected and non-ruminant-derived DNAs were not detected with 100% accuracy. Thus, it is clear that the combination of primers employed significantly affects ruminant detection accuracy, where the claimed primer pair can specifically detect ruminant-derived DNA without false positive or negative results.

Therefore, it would not have been obvious to those skilled in the art to expect the claimed primers to have the unexpected property of accurate ruminant-derived DNA detection. That is, one skilled in the art could not predict that any of the primer sequences which are designed based on the boxed areas in Appendix 2 would have the functionality for accurate ruminant-derived DNA detection.

I, Koichi Kadowaki, hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued therein.



Koichi Kadowaki, Ph.D.

Dec. 18, 2009

Date

試料に混在する成分の動物種を同定できます

BIOFOOD Kit

生加工および加工済みの食品や飼料などの試料に存在する動物由来成分を検出し、動物種を同定するキットです。試料から精製した DNA を、PCR で増幅後、アガロースゲル電気泳動で検出します。
※キットに DNA 精製用試薬は含まれません。

試料の種類には「食品・飼料用試薬」や遺伝子分離による「PCR用試薬」が便利です。

目録表

- ・ BIOFOOD Identification Kit では、製品表示以外の肉類の混入の有無を検査し、またその動物種を同定することができます。
- ・ タンパク質の抽出を行うキットと異なり、試料中の DNA を検出するため、洗剤、化学的・物理的処理、粉砕、凍結、加熱、凍結などの処理をした後の試料からも検出できます。
- ・ キットには、PCR による増幅に必要な試薬およびコントロール用 DNA が含まれており、PCR の感度性や、反応系への PCR 阻害物の混入を検査できます。

I BIOFOOD Standard Kit

許容動物(豚、鶏、牛、馬、魚)に共通な配列に対するプライマーを用いた PCR を行い、これらの動物由来成分の存在の有無を検査するキットです。スクリーニングに適しています。
感度: 0.5 % 以上

II BIOFOOD Identification Kit

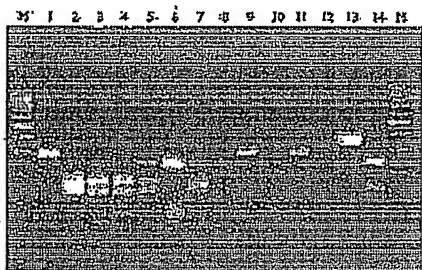
試料中の動物由来成分の動物種を同定するキットです。許容動物に共通な配列に対するプライマーを用いて PCR を行った後、3 種類の制限酵素で切断し、RFLP (Restriction Fragment Length Polymorphism: 制限酵素断片長多型) の電気泳動パターンにより動物種を同定します。
感度: 0.5 ~ 5 % 以上

※ 5 % 以上の動物由来成分が混在した試料の検査では、結果の判読が困難な場合があります。

III BIOFOOD Mixup Kit

6 種類の動物(ウマ、ヒツジ、ウシ、ブタ、ニワトリ、ヤギ)それぞれに特異的なプライマーの混合物を用いた PCR により、これらの動物由来成分の有無を検査するキットです。上記のキットなどで既に検査を行った試料に対して、簡便に再検査できます。
感度: 2 % 以上

※ 検出された動物由来成分が混在した試料の検査には適していません。



RFLP 分析例

様々な試料から精製した DNA を PCR で増幅後、BIOFOOD Identification Kit の Enzyme 1 で切断し、アガロースゲル電気泳動を行った。キットに含まれる別の制限酵素で処理した場合の結果と組み合わせることにより、動物種を特定できる。

- lane 1: スイギュウ
- lane 2: ウサギ
- lane 3: ウマ
- lane 4: ノロジカ
- lane 5: ウシ
- lane 6: ヤギ
- lane 7: シカ
- lane 8: エミュー
- lane 9: アガカンガル
- lane 10: タチウ
- lane 11: キコ
- lane 12: イヌ
- lane 13: マウス
- lane 14: ヒト
- lane M: 100 bp サイズマーカー

キット内容

試薬

・ $MgCl_2$ solution

Appendix 1

- DNA polymerase

Standard Kit

- Master mix
- Mixed control material

Identification Kit

- Control material
- Master mix
- Restriction enzyme 1, 2, 3
- Enzyme buffer 1, 2, 3

Mixed Kit

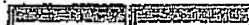
- Multiplex master mix
- Processed control material
- Control DNA

目録 表

在庫：2009年09月30日 11:15 現在

メーカーコード	商品コード	品名	包装	価格(円)	在庫	保存条件	詳細情報
		説明文				延焼回数	
		総販売力ログ					
ETL	91.111	BIOFOOD Standard Kit (24 tests)	1kit	45,000	お問合せ		
<input type="checkbox"/>		この製品の取扱は終了いたしました。					
ETL	91.112	BIOFOOD Standard Kit (48 tests)	1kit	58,000	お問合せ	-20℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。					
ETL	91.121	BIOFOOD Identification Kit Standard Control (Chicken, Goat, Pig, Turkey) (24 tests)	1kit	49,000	お問合せ	-20℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。					
ETL	91.122	BIOFOOD Identification Kit Standard Control (Chicken, Goat, Pig, Turkey) (48 tests)	1kit	62,000	お問合せ	-20℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。 ニュース 2007年10月15日号(No.450) (p. 5)					
ETL	91.131	BIOFOOD Mixed Kit (24 tests)	1kit	49,000	お問合せ		
<input type="checkbox"/>		この製品の取扱は終了いたしました。					
ETL	91.132	BIOFOOD Mixed Kit (48 tests)	1kit	62,000	お問合せ	-20℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。					

>> 本文書内容を見る



ページトップへ

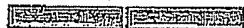
Genomic DNA Extraction Kit

在庫：2009年09月30日 11:15 現在

メーカーコード	商品コード	品名	包装	価格(円)	在庫	保存条件	詳細情報
		説明文				延焼回数	

CTL	91.011	Control DNA, Human, BIOFOOD Identification	500ng	14,000	お問合せ	4℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。 総合カタログ2005-2006[試験用]製品リスト (p. 249) 遺伝子工学 研究用試薬・検器 2004 (p. 461) ニュース 2003年11月1日号 (No.303) (p. 7)					
CTL	91.014	Control DNA, Human, BIOFOOD Identification	500ng	14,000	お問合せ	4℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。 総合カタログ2005-2006[試験用]製品リスト (p. 249) 遺伝子工学 研究用試薬・検器 2004 (p. 461) ニュース 2003年11月1日号 (No.303) (p. 7)					
CTL	91.014	Control DNA, Pig, BIOFOOD Identification	500ng	14,000	お問合せ	-20℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。 総合カタログ2005-2006[試験用]製品リスト (p. 249) 遺伝子工学 研究用試薬・検器 2004 (p. 461) ニュース 2003年11月1日号 (No.303) (p. 7)					
CTL	91.010	Control DNA, Rabbit, BIOFOOD Identification	500ng	14,000	お問合せ	4℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。 総合カタログ2005-2006[試験用]製品リスト (p. 249) 遺伝子工学 研究用試薬・検器 2004 (p. 461) ニュース 2003年11月1日号 (No.303) (p. 7)					
CTL	91.008	Control DNA, Sheep, BIOFOOD Identification	500ng	14,000	お問合せ	-20℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。 総合カタログ2005-2006[試験用]製品リスト (p. 249) 遺伝子工学 研究用試薬・検器 2004 (p. 461) ニュース 2003年11月1日号 (No.303) (p. 7)					
CTL	91.007	Control DNA, Turkey, BIOFOOD Identification	500ng	14,000	お問合せ	-20℃	
<input type="checkbox"/>		この製品の取扱は終了いたしました。 総合カタログ2005-2006[試験用]製品リスト (p. 249) 遺伝子工学 研究用試薬・検器 2004 (p. 461) ニュース 2003年11月1日号 (No.303) (p. 7)					

>> 注文書内容を見る



ページトップへ

ご購入時のご注意

BIOFOOD Identification Kitは、キットに添付するコントロール DNA を4種ご指定いただくことができます。コントロールの指定をご希望の場合は、製品は本文でのご注文となります。注文時に必要事項をご記入の上、販売店にお送り下さい。なお、ご指定がない場合は、4種(chicken, goat, pig, turkey)を組み合わせた標準セットになります。詳細は、下記までお問い合わせ下さい。

[電話] 03-5884-1020 [FAX] 03-5884-1775

お問い合わせ先

テクニカルサポート(販促担当)

[TEL] 03-5884-1020 [FAX] 03-5884-1775

製品情報は掲載時点のもですが、価格表内の価格については随時最新のものに変更されます。お問い合わせいただいたタイミングにより製品情報・価格などは変更されている場合があります。表示価格に、消費税は含まれていません。一部価格が予告なく変更される場合がありますので、あらかじめご了承ください。

サイトマップ | サイトのご利用方法 | プライバシーポリシー | 送料方法 | 製品取扱上の注意

Copyright (C) 2008 Fumakoshi Corporation, All Rights Reserved.

Partial Translation of Appendix 1
(<http://www.funakoshi.co.jp/node/10898>)

Translation of the section "BIOFOOD Identification Kit"

--BIOFOOD Identification Kit

This kit is to identify animal species for animal-derived components in a sample. First, the primer pair against the sequence common to vertebrates is used to perform PCR, and then the amplified DNA is digested with three restriction enzymes and subjected to gel electrophoresis. Animal species for animal-derived components in the sample are identified based on the electrophoresis patterns of RFLP (Restriction Fragment Length Polymorphism).

Sensitivity: 0.5% to %% or more

*For a sample containing components derived from three or more of animal species, it may be difficult to give an electrophoresis pattern.

Translation of the legend to photograph of RFLP pattern (Fig. 1 above)

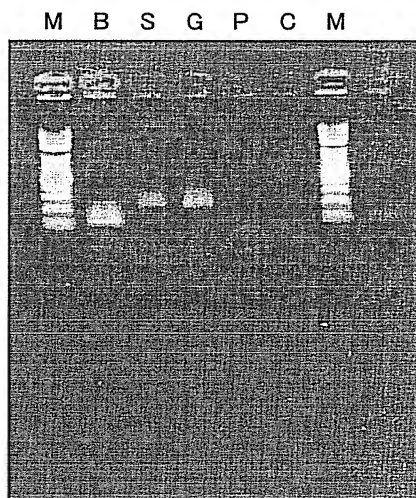
Example of RFLP analysis

DNA was purified from various samples, amplified by PCR, and then digested with Enzyme 1 of BIOFOOD Identification Kit and subjected to agarose gel electrophoresis. By combining this result with the other results obtained by using the other restriction enzymes included in the Kit, animal species for animal-derived components in the sample can be identified.

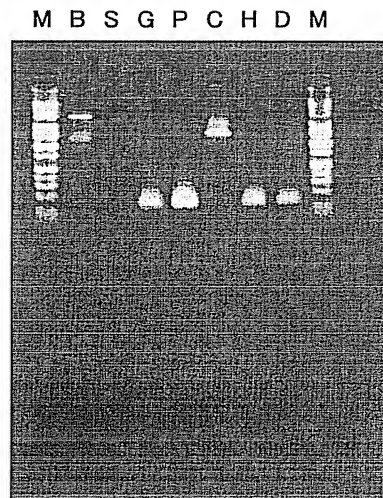
Lane 1: buffalo; Lane 2: rabbit; Lane 3: horse; Lane 4: roe deer; Lane 5: cattle; Lane 6: Goat; Lane 7: deer; Lane 8: emu; Lane 9: red kangaroo; Lane 10: ostrich; Lane 11: cat; lane 12: dog; lane 13: muse; lane 14: human; lane M: 100 bp size marker.

Appendix 3

Case 1



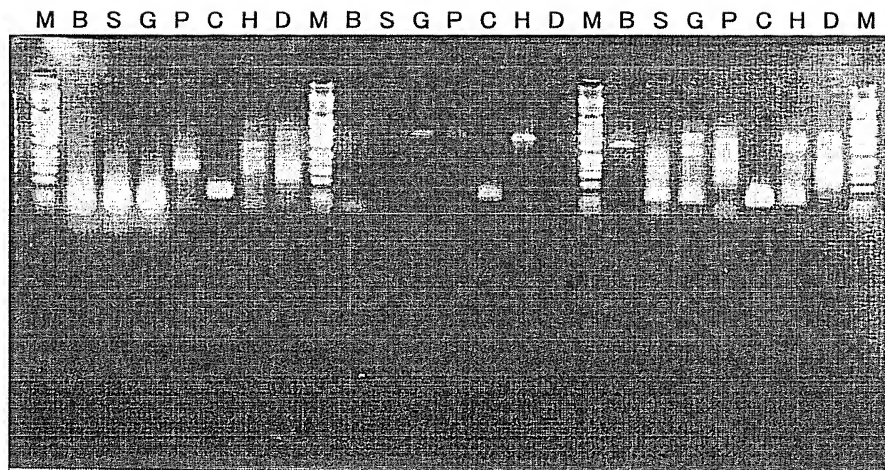
Case 2



Case 3

Case 4

Case 5



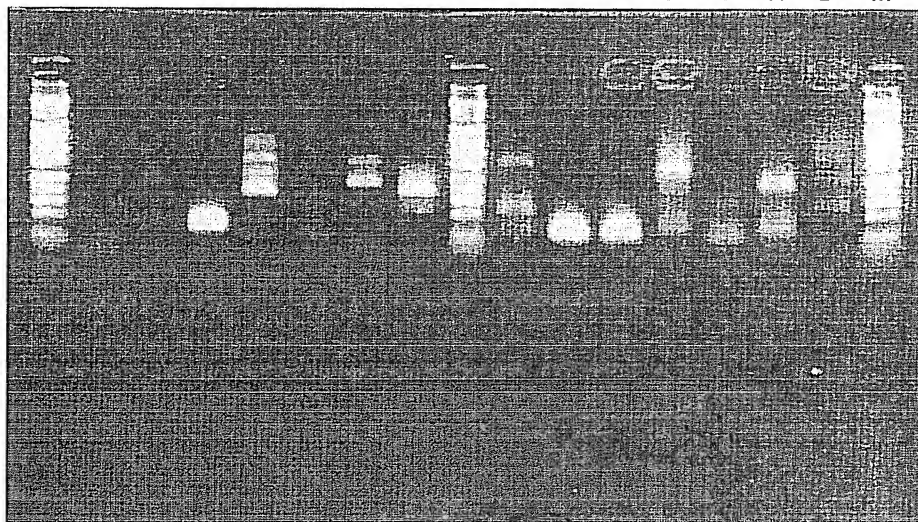
M: 100 bp DNA size markers

B: Cattle, S: Sheep, G: Goat, P: Pig, C: Chicken, H: Horse, D: Deer

Case 6

Case 7

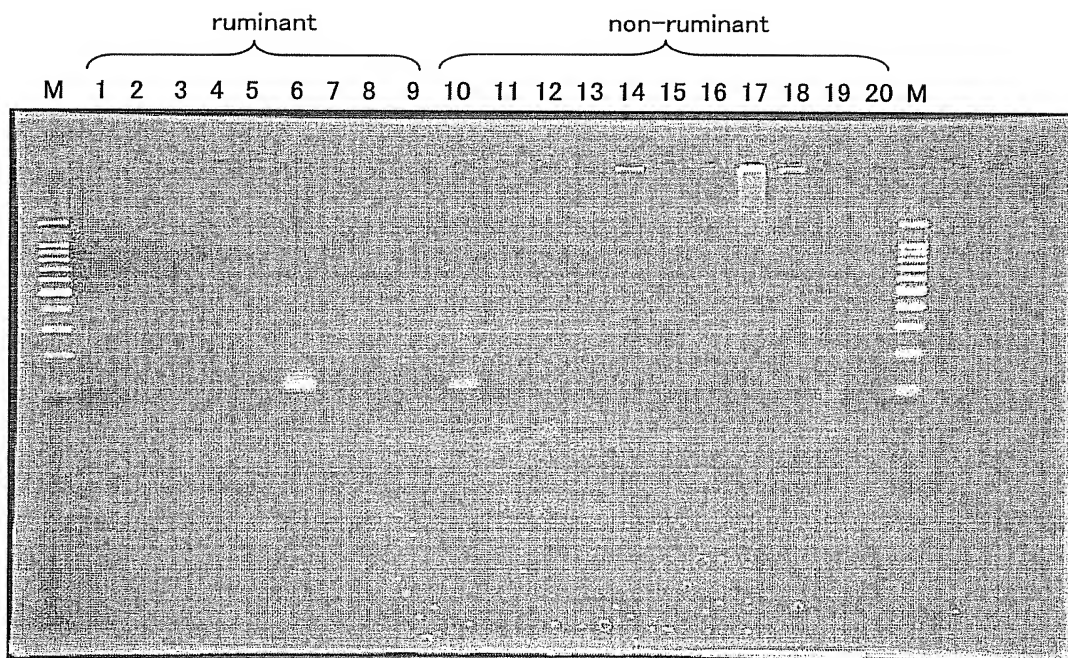
M B S G P C H D M B S G P C H D M



M: 100 bp DNA size markers

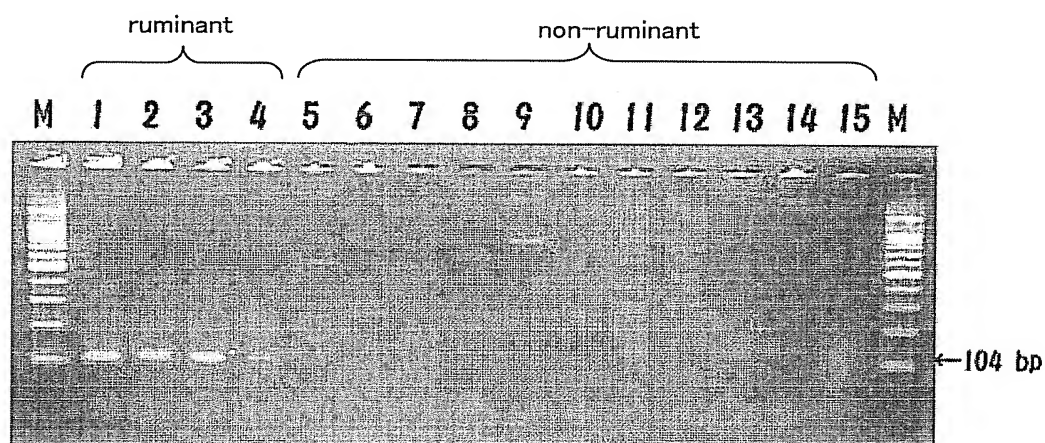
B: Cattle, S: Sheep, G: Goat, P: Pig, C: Chicken, H: Horse, D: Deer

Case 8



1-5: Cattle, 6: Sheep, 7: Goat, 8-9: Deer,
10: Swine, 11: Horse, 12: Rabbit, 13: Whale, 14: Poultry, 15: Codfish,
16: Salmon, 17: Sardine, 18: Crab, 19: Prawn, 20: Shellfish,
M: 100 bp DNA size markers

rumicon (claimed primer pair): same as Fig. 7 in the specification



1: Cattle, 2: Sheep, 3: Goat, 4: Deer,
5: Swine, 6: Horse, 7: Rabbit, 8: Whale, 9: Poultry, 10: Codfish,
11: Salmon, 12: Sardine, 13: Crab, 14: Prawn, 15: Shellfish,
M: 100 bp DNA size markers